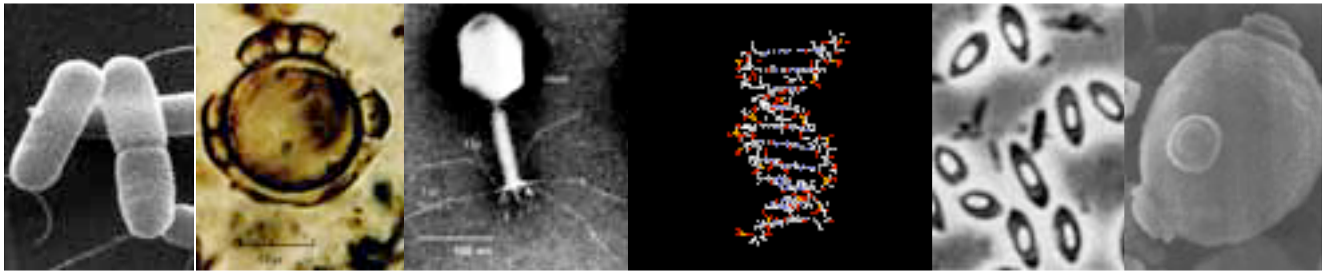


Microbial metabolism
Part 1.

General Microbiology – Lecture 6

Cañada College – Fall 2006

Instructor: Tamas Torok, Ph.D.

Berkeley Lab

Topics for the day

- Metabolism in general
- Enzymes
- Energy production/catabolism
  - substrate-level phosphorylation
    - glycolysis and alternatives
    - fermentation

Metabolism

- Metabolism
  - sum total of all reactions that occur in a cell
- Catabolic reactions
  - break down of complex molecules into smaller, simpler molecules with the release of energy and reducing power (electrons)
- Anabolic reactions
  - synthesis of complex molecules from simpler ones
  - requires energy and reducing power (electrons) to form cell structures
- Catabolic and anabolic reactions
  - coupled, highly regulated, interdependent, and ***simultaneous***

Basic energy concept

- Cells
  - open, non-equilibrium systems
- First law of thermodynamics
  - energy can neither be created nor destroyed in the universe
- Second law of thermodynamics
  - in any reaction the amount of molecular disorder always increases

Energy production

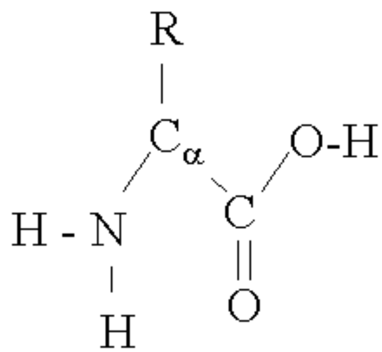
- Redox reaction
  - oxidation: loss of electrons
  - reduction: gain of electrons
  - reduction potential (E_0)
  - half reactions
    - H_2 —> $2H^+ + 2e^-$ (E_0'= −0.42V)
    - 1/2 $O_2 + 4\ e^-$ —> H_20 (E_0'= +0.82V)
    - $\Delta G^{0\prime} = (-nF)(\Delta E_0') = (-2)(-96.48kJ/V)(+0.82V-0.42V) = 239.27\ kJ$
  - each molecule has the potential to donate and accept electrons from another molecule

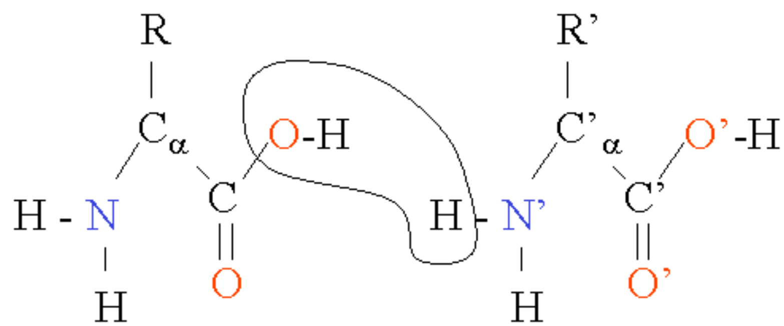
Table 8.1 Half reactions, the number of electrons transferred (*n*), and the electrode potential under standard conditions (E_o') compared to the hydrogen half cell

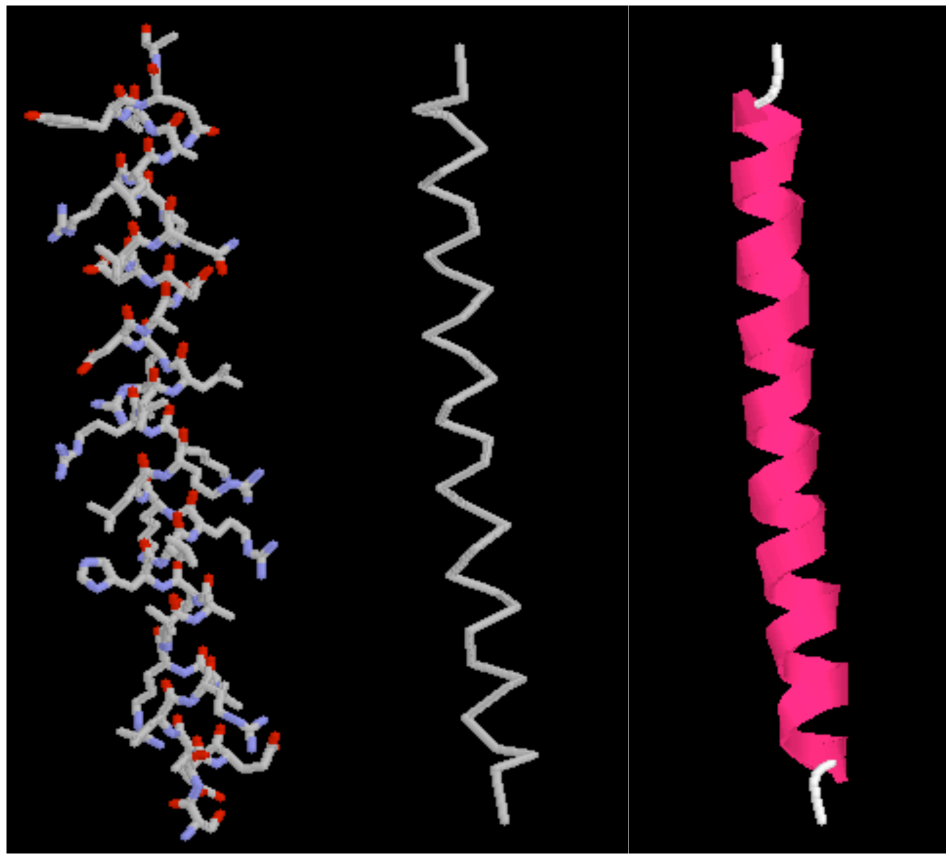
Half Reaction	*n*	E_o' (V)
Ferredoxin (oxidized/reduced)	2	–0.43
$2\ H^+/H_2$	2	–0.42
$NADP^+ + H^+/NADPH + H^+$	2	–0.32
1,3-di-P-glycerate + $2H^+$/ glyceraldehyde-3-P + P_i	2	–0.29
Chlorophyll (P_{II})	1	–0.20
$FMN + 2H^+/FMNH_2$	2	–0.22
$FAD + 2\ H^+/FADH_2$	2	–0.22
Standard half cell $2\ H^+/H_2$	**2**	**0.00**
Methylene blue (oxidized/reduced)	2	+0.01
Fumarate + $2H^+$/succinate	2	+0.03
Ubiquinone (oxidized/reduced)	2	+0.06
Cytochrome *b* (Fe^{3+}/Fe^{2+})	1	+0.08
Cytochrome *c* (Fe^{3+}/Fe^{2+})	1	+0.25
Chlorophyll (P_I)	1	+0.40
$NO_3^- + 2\ H^+/NO_2^- + H_2O$	2	+0.42
Fe^{3+}/Fe^{2+}	1	+0.77
$2\ H^+ + \frac{1}{2}\ O_2/H_2O$	2	+0.82

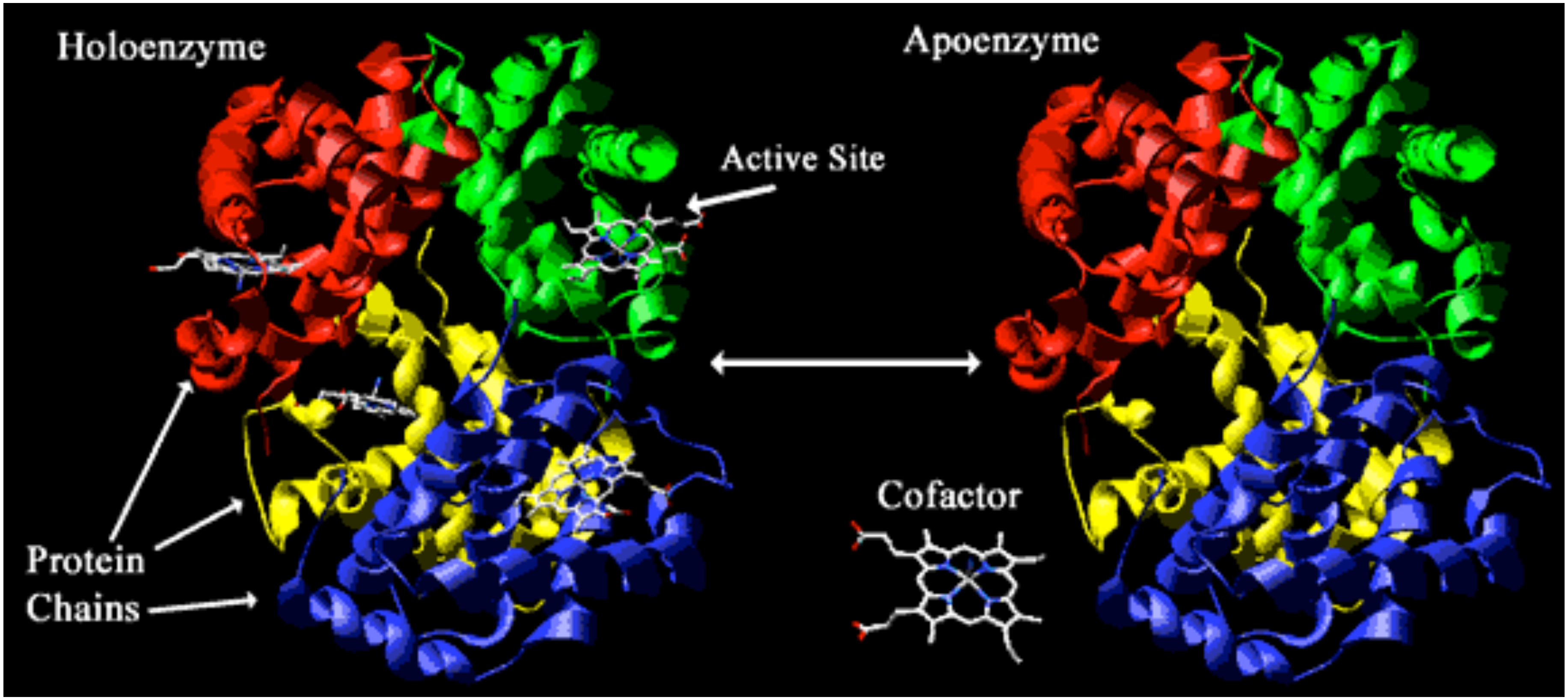
Enzymes

- What is an enzyme?
- Functional enzyme
- Mechanism of enzymatic reaction
  - "generic" version
  - one enzyme – many substrates
  - many enzymes – one substrate
  - classes of enzymes
- Regulation of enzymes
  - via synthesis (topic for a later evening)
  - via activity








Holoenzyme
Apoenzyme
Active Site
Protein
Chains
Cofactor

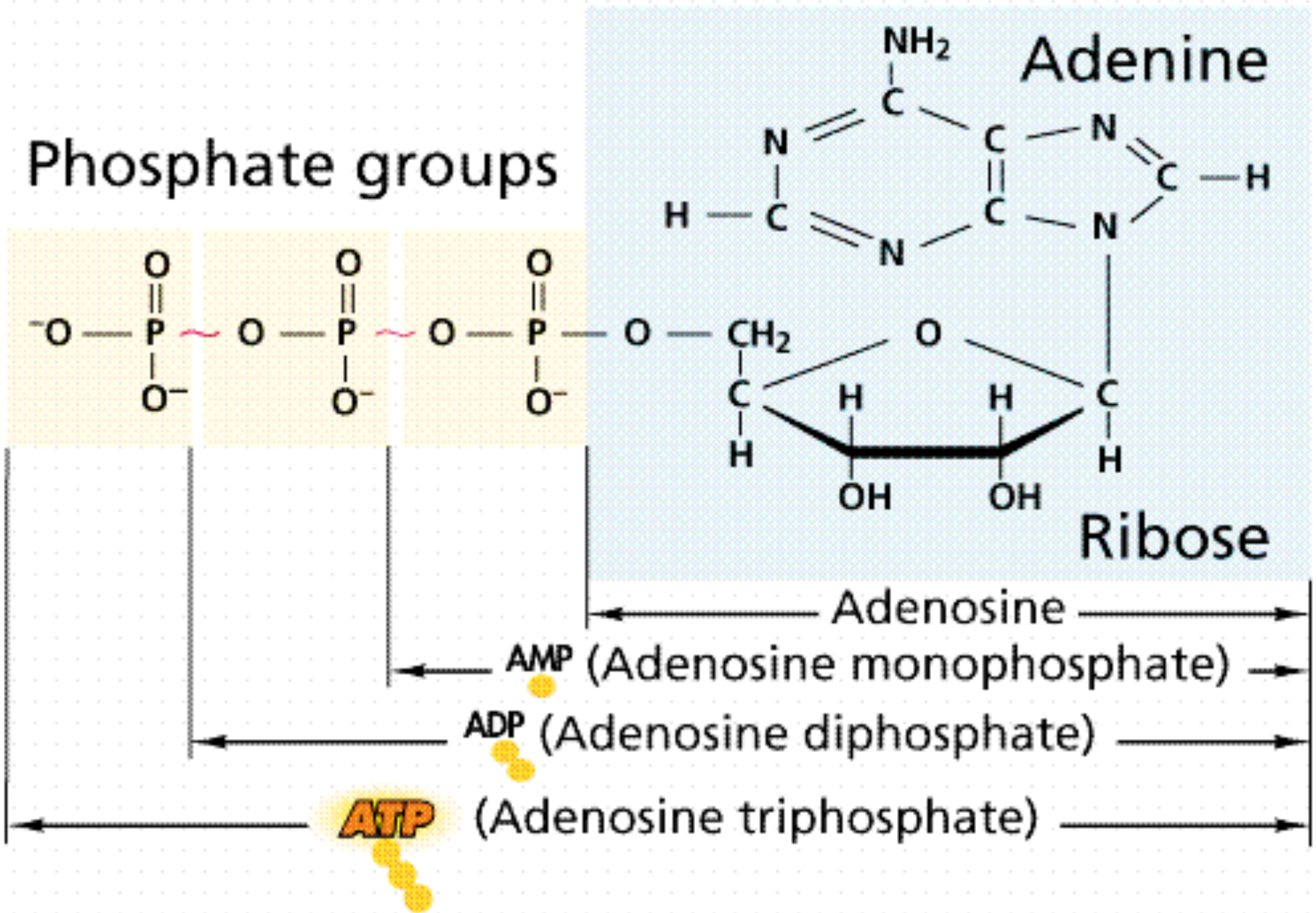
Factors influencing enzyme activity

- Temperature
- pH
- Pressure
- Substrate concentration
- Post-translational regulation
  - inhibitors
    - competitive inhibition
    - allosteric inhibition
  - feedback inhibition

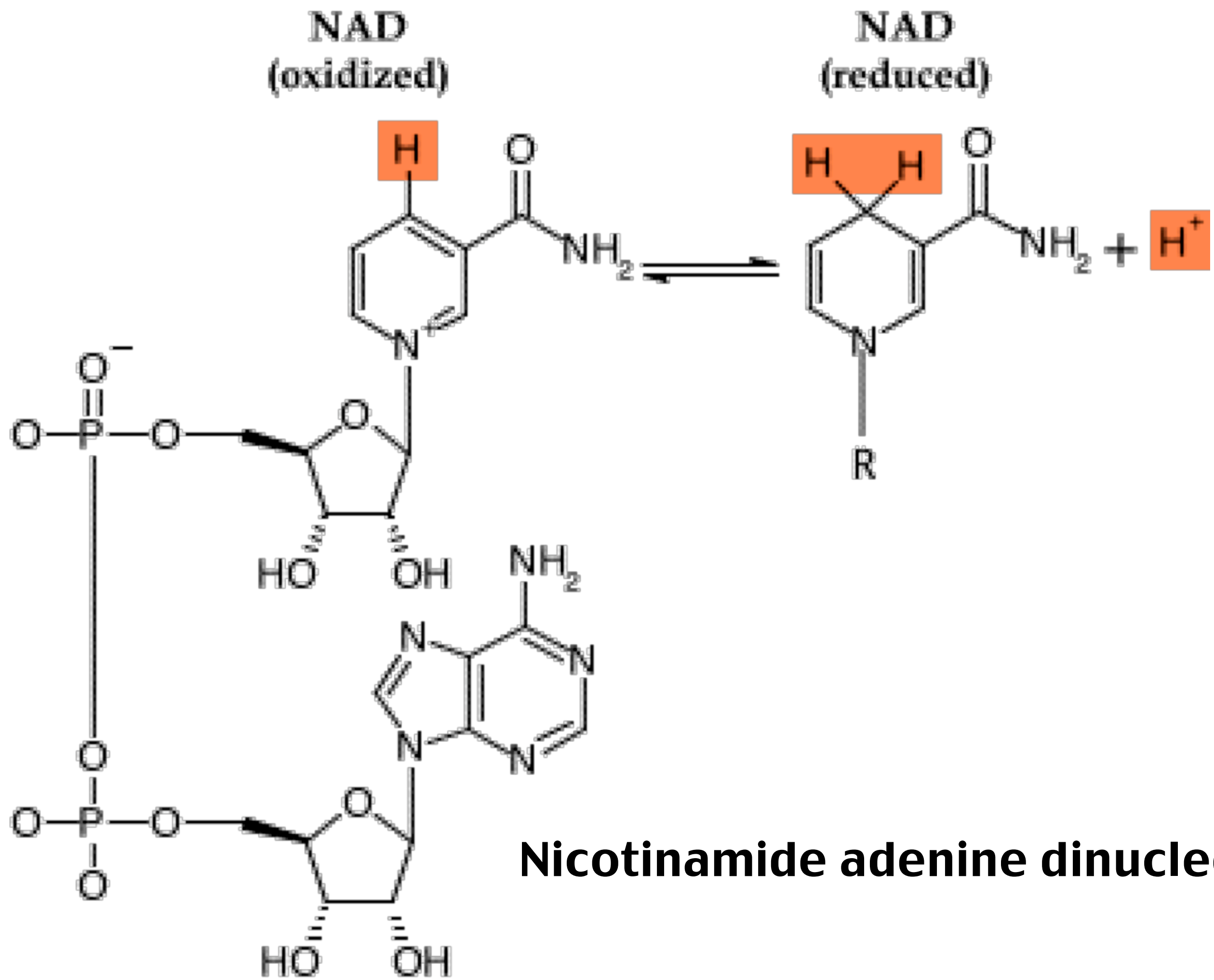
Catabolism

- Goal
  - generate energy carriers (ATP, GTP) and electron carriers (NAD and FAD)
- Energy and reducing power fuel growth, repair, cell maintenance, and movement

ATP (adenosine triphosphate)



NAD/NADH



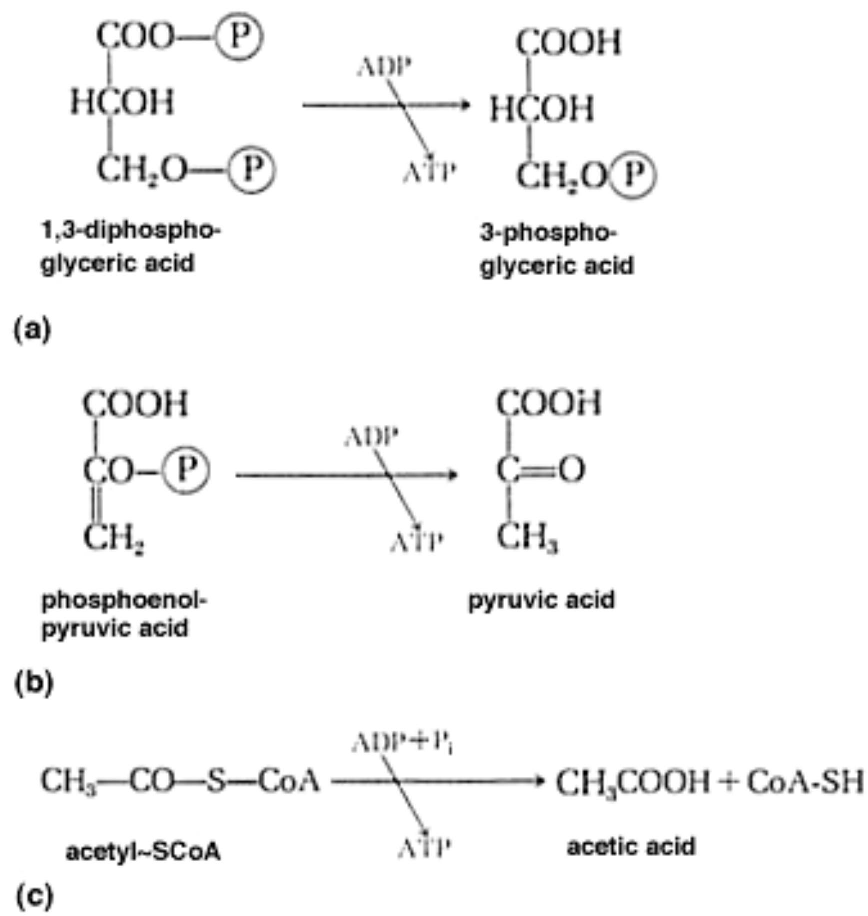
Nicotinamide adenine dinucleotide

Energy production

- ATP generation
  - substrate-level phosphorylation (SLP)
  - oxidative phosphorylation (ETLP)
  - photo-phosphorylation

Substrate-level phosphorylation

- SLP
  - synthesis of ATP directly coupled to the breakdown of high energy organic substrates

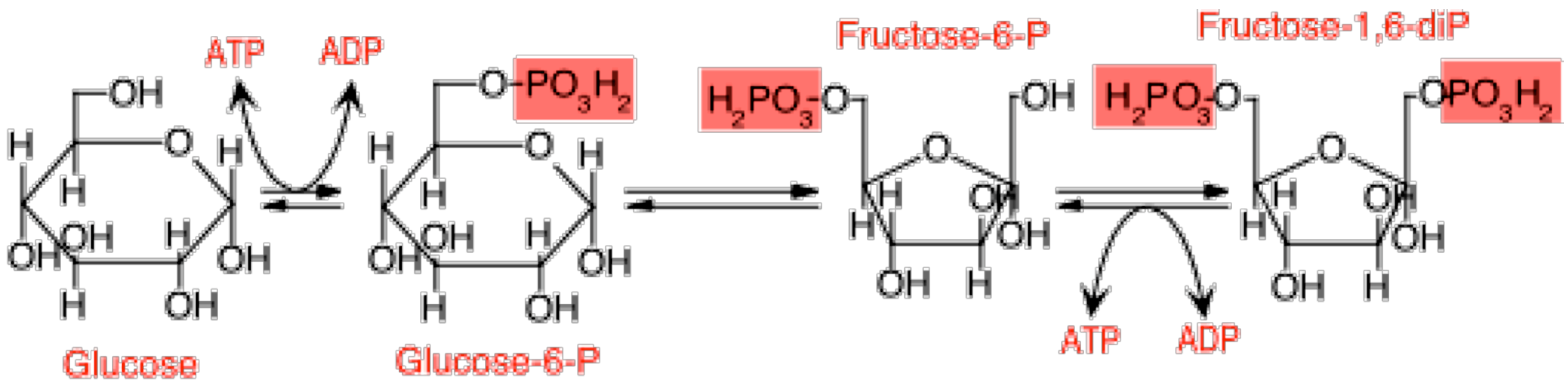


Glycolysis
(Embden–Meyerhoff–Parnas pathway)

- Most commonly used series of reactions for oxidizing glucose to pyruvate
- Glycolysis can occur in the presence or absence of oxygen
- Net gain of 2 ATP and 2 NADH (reduced electron carrier) molecules

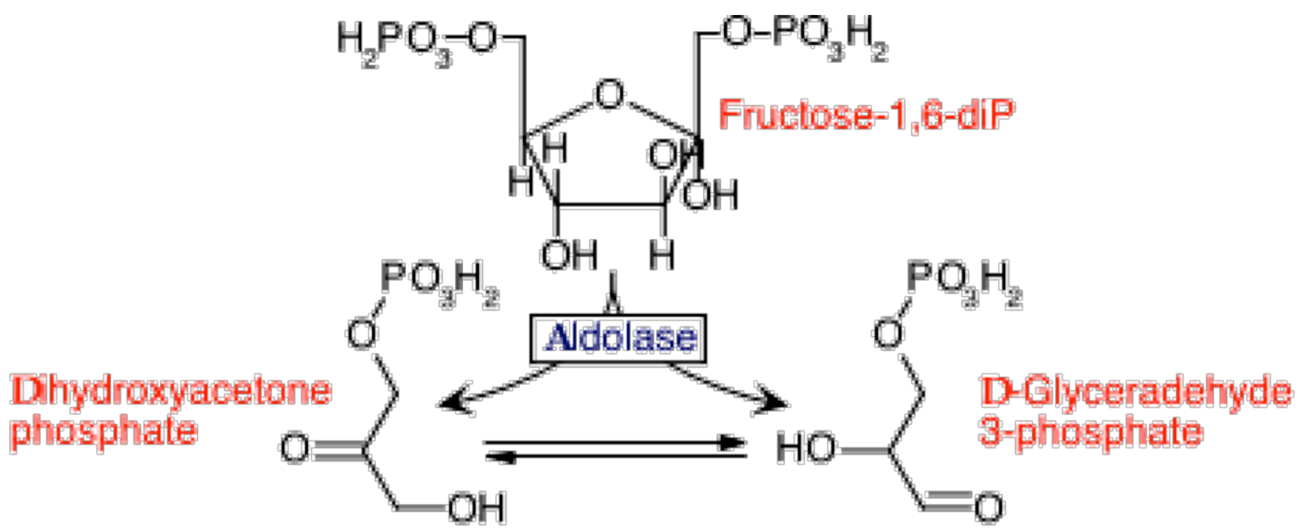
Glycolysis (cont.)

- **Activation of glucose**



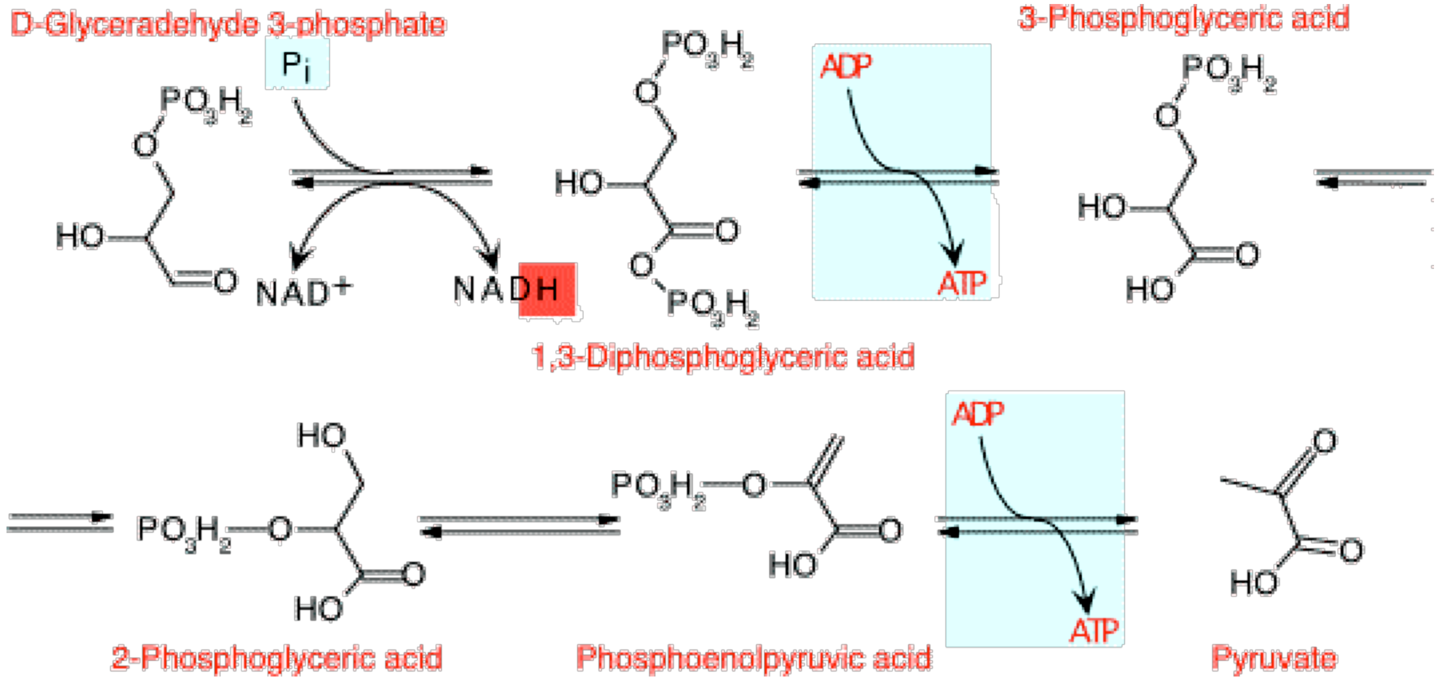
Glycolysis (cont.)

- Hexose splitting

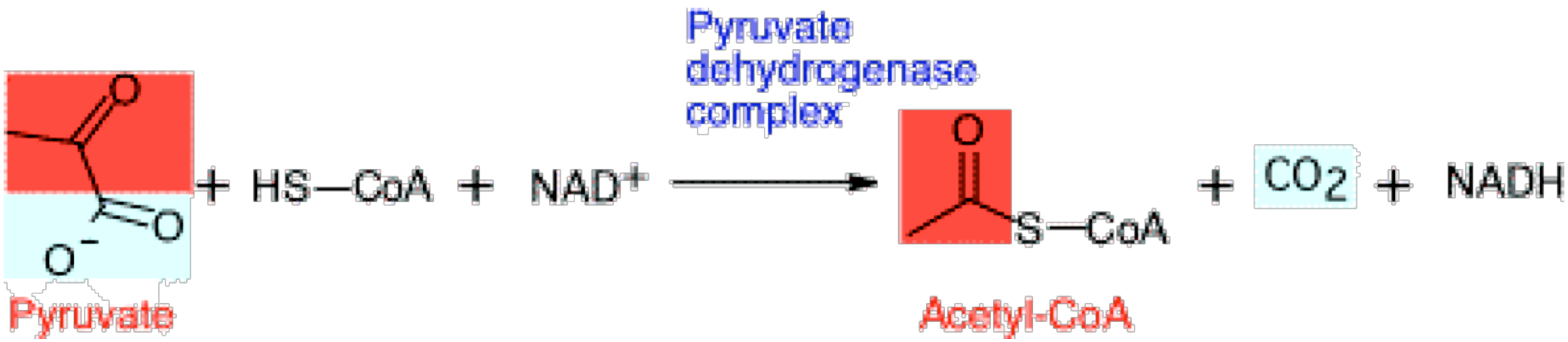


Glycolysis (cont.)

- **Energy extraction**



Coupling glycolysis to respiration



Alternatives to glycolysis

- Pentose phosphate pathway
- Entner–Dudoroff pathway

Pentose phosphate pathway

- Uses the 6 carbons of glucose to generate 5 carbon sugars and reducing equivalents (oxidative and non-oxidative branches)
- Under certain conditions it can completely oxidize glucose to CO_2 and water
- Operates exclusively in the cytosol
- Primary functions
  - generates reducing equivalents, NADPH, for reductive biosynthesis
  - provides the cell with ribose-5-phosphate (R5P) for the synthesis of the nucleotides and nucleic acids
  - metabolizes pentose sugars derived from the digestion of nucleic acids
  - rearranges the carbon skeletons of carbohydrates into glycolytic/gluconeogenic intermediates

Entner–Dudoroff pathway

- **Only a few bacteria, e.g. *Zymomonas*, employ the Entner–Doudoroff pathway as a *fermentation* path**
- **Many bacteria, especially pseudomonads, use the pathway to degrade carbohydrates for *respiratory* metabolism**
- **Entner–Dudoroff pathway yields 2 pyruvic acid from glucose (same as glycolysis)**
- **Oxidation occurs before the cleavage, and the net energy yield per mole of glucose used is one mole of ATP**

Fermentations

- Alternative to respiration
- Goal
  - NADHs need to be oxidized, “recycled”
  - pyruvate converted
- Examples
  - lactic acid fermentation
  - alcohol fermentation
  - heterofermentative microbes